EVIDENCE FOR THE EXISTENCE OF HOMOZYGOUS CLONES IN THE SELF-FERTILIZING HERMAPHRODITIC TELEOST RIVULUS MARMORATUS (POEY)[1]

KLAUS D. KALLMAN AND ROBERT W. HARRINGTON, JR.

Genetics Laboratory, New York Aquarium, New York Zoological Society, American Museum of Natural History, New York 24, N. Y., and Entomological Research Center, Florida State Board of Health, Vero Beach, Florida

The prevalence of sexual reproduction among animals and plants attests to the great adaptive value of this mechanism. Members of sexually reproducing populations share a common gene pool from which a virtually unlimited number of genetic recombinations can be obtained through cross-fertilization, thus achieving the genetic variability that in the long run enables species to survive environmental change. There is also evidence that in Mendelian populations the heterozygous condition is superior in fitness to the homozygous one, heterozygous individuals showing greater adaptability to environmental variables.

Uniparental reproduction, on the other hand, prevents a species or race from acquiring new genotypes through recombination, and mechanisms such as parthenogenesis, gynogenesis and self-fertilizing hermaphroditism have been considered to lead into evolutionary blind alleys. Uniparental reproduction, to be sure, may be of temporary advantage in that favorable gene combinations can be maintained and rapidly passed on to future generations. All races and species that normally reproduce by these three mechanisms are composed of clones, the members of which have identical genotypes. In ameiotic parthenogenesis, the eggs do not undergo reduction divisions and the individuals arising from them are heterozygous, the degree of heterozygosity steadily increasing as gene and chromosome mutations accumulate. If this goes to the extreme, the two sets of chromosomes eventually become so dissimilar that the genome can no longer be considered diploid (White, 1954). In meiotic parthenogenesis the offspring may have arisen from haploid eggs, the diploid condition being reestablished either by the fusion of the egg nucleus with the second polar body nucleus or by the suppression of the first cleavage division. The first pathway leads to homozygosity within a few generations while the latter results in complete homozygosity in a single step. Hermaphroditism, when coupled with self-fertilization, results in a sharp decline in heterozygosity, leading to a population of homozygous individuals within 7 to 10 generations—in fact it constitutes the ultimate mode of inbreeding.

Several types of parthenogenesis are of regular occurrence in certain groups of invertebrates (Suomalainen, 1962; White, 1954), but well-documented cases of uniparental reproduction in vertebrates are extremely rare and each instance

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deserves special attention. A few teratological cases of self-fertilization resulting in uniparental offspring have been described in the guppy, *Lebistes reticulatus* (Spurway, 1957; Comfort, 1961), and Kilby and Kallman (unpublished) observed several cases of parthenogenesis in the mosquito fish, *Gambusia affinis*. Parthenogenesis has been found sporadically in a race of the domesticated turkey (for summary see Olsen, 1962).

Parthenogenesis occurs normally in several all-female subspecies of the Armenian lizard, *Lacerta saxicola* (Darewski and Kulikowa, 1961), and is suspected to occur in several other species of lizards in the genus *Cnemidophorus* (Maslin, 1962). In fishes, Hubbs and Hubbs (1932, 1946) discovered that *Mollienesia formosa* exists only as females, which reproduce by gynogenesis following insemination by males of related species. The sperm merely activates the eggs without contributing any genetic material. Gynogenesis has also been reported to occur in certain populations of the cyprinid *Carassius auratus gibelio* (Lieder, 1955).

Confusion exists in the literature with respect to the occurrence of hermaphroditism in fishes. In contrast to the assertions found in many recent text books and reviews, that the Sparidae are functional hermaphrodites, Reinboth (1962) points out that in the order Perciformes simultaneous functional hermaphroditism is restricted to certain species of serranids, protogyny occurs in some serranids, sparids, centracanthids and labrids while protandry is found in certain sparids. Among normally hermaphroditic perciforms, only in serranids are ovarian and testicular portions of the gonad active simultaneously. The eggs and sperm are emitted through separate ducts, and self-fertilization has been obtained experimentally in *Serranus scriba* (Reinboth, 1962; Salekhova, 1963) and in *S. subligarius* (Clark, 1959). Nevertheless, observations by all three investigators, in large-sized aquaria or in the natural habitat, indicate that spawning activity is initiated between two or more individuals, and it seems likely that cross-fertilization is the rule.

With the recent discovery of *Rivulus marmoratus* (Poey) along the Florida East Coast (Harrington and Rivas, 1958) and the subsequent finding that all collected specimens tested and most of their known descendants were functional, consistently self-fertilizing, oviparous hermaphrodites (Harrington, 1961, 1963), the possible homozygosity of these hermaphrodites and of their uniparental descendants came into question. The present report concerns the unique genetic relationship existing between normal self-fertilizing parents and their offspring. The tissue transplantation test (Kallman, 1962) has been used to determine whether parent and offspring have identical genotypes.

Material and Methods

Six self-fertilizing hermaphroditic *R. marmoratus*, collected in 1961 in their natural habitat near Vero Beach, Florida, and their descendants were used in the experiments described below. From the time of their capture the wild fish were kept isolated in small aquaria, and all future generations originated from individuals raised *ab ovo* in individual glass jars. Each egg thus allocated to its own rearing jar was obtained at the precise moment it was emitted (oviposited) by its hermaphrodite parent (for details, see Harrington, 1963). The six progenitors were designated *FT*, *NA*, *DS*, *NL*, *NSU* and *NSB*.

For ready identification of individual fish the following system has been adopted. The first letter following the hyphen always identifies fish of the first laboratory generation, the second and third letters identify the fish of the second and third generations, respectively. Thus *DS-ACH* is a third generation fish, "H," the offspring of second generation fish "C" which in turn was derived from fish "A" of the F_1 generation. The original progenitor was *DS*.

Anal, dorsal and caudal fins, hearts and spleens were transplanted according to a method described previously (Kallman and Gordon, 1958; Kallman, 1960). These structures were selected, because they can easily be grafted and their fate readily ascertained. Inadequate numbers of fish in certain lineages made it necessary to take more than one graft from the same donor and to give some hosts two transplants, from different donors. To increase the number of grafts that could be obtained from a fish, the caudal fin of the donor was often split into two halves along the midlateral plane and the spleen (in large donors), divided into two parts. In grafting, the transplant is inserted into a slitlike pocket cut into the musculature of the caudal peduncle. The suspensorium of the fin graft is pushed into the pocket with a blunt needle, while the external portion of the fin protrudes from the mouth of the pocket. As a consequence of being denervated, the transplanted fin initially undergoes degeneration, starting at its distal end, but six to eight days later, upon reinnervation, regeneration ensues. Spleen and heart grafts are pushed into similar pockets. Grafts are prevented from falling out by muscle contraction around the pocket. Nevertheless, two days after the operation all hosts were examined under a dissecting microscope to learn whether any graft had been lost for mechanical reasons.

Attempts to transplant scales according to the method of Hildemann (1957) proved impractical for two reasons. The scales of *Rivulus* are small and delicate and the integument is covered with a heavy layer of mucus that makes it difficult to insert a scale graft into a scale pocket of the host. In these fish, it is also difficult to distinguish a successfully transplanted scale from the many scales of the host that are similar in size and color.

The age of laboratory-reared donors ranged from 20 to 163 days post-hatching and that of the hosts from 34 to 536 days. In addition, one donor (NSU) and two hosts (FT, NA) were brought in from the wild already fully mature sexually and were perhaps about a year old. All fish were maintained in isolation from other fish, in 40% sea water (distilled water and filtered sea water) at temperatures ranging from 21° C. to 29° C. and averaging 25° C.; they were fed on brine shrimp nauplii and mosquito larvae (*Aedes* sp.). Their solid wastes and uneaten food were siphoned out each day; the water was filtered once a week, and changed completely at the first signs of cloudiness. With the exception of a single fish that died within three months after the operation, all hosts were maintained for seven months or longer.

Results

Intra-sib grafts were made in 26 different host-donor combinations (Table I) to determine whether sibs possess identical genotypes. Five hosts (#8, 10, 14, 16, 18) each received two grafts from the same donor to bring the total number of intra-sib grafts to 31. In these five combinations, the hosts were of appreciably

larger size than the donors, the dorsal fin of the donor being much smaller than the scales of the host, and it was feared that because of their small size the grafts might become damaged or might be resorbed, even when host and donor possessed compatible genotypes. Two grafts were therefore implanted into the hosts in the hope that at least one of the grafts would fulfil the surgical requirements for survival.

TABLE 1

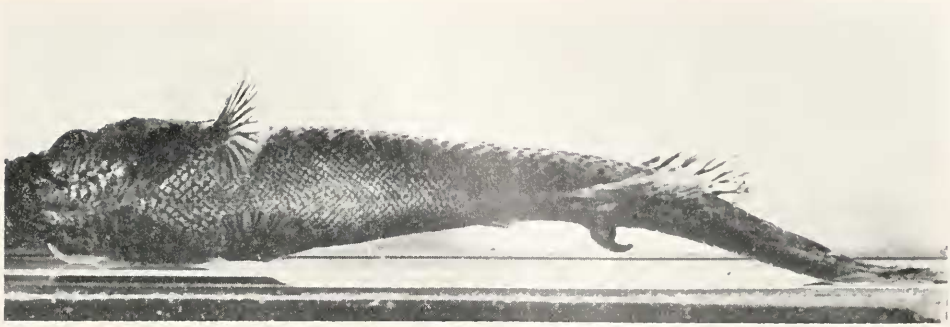
Fate of intra-sib grafts in Rivulus marmoratus

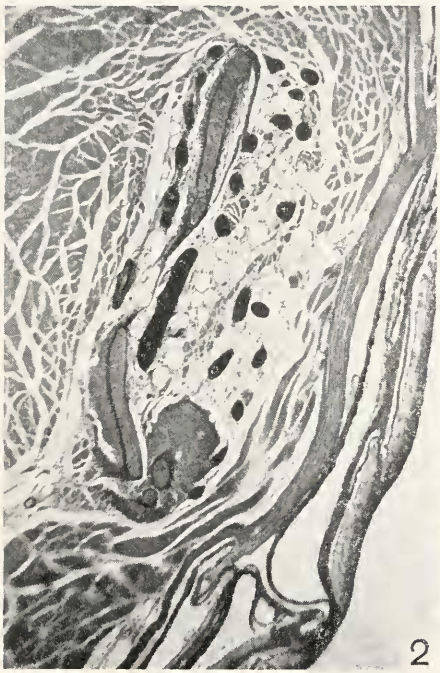
	Donor	Host	Age of host	Type of graft	Fate of graft	Criteria of graft survival	Time (days)
1	*FT*-m	*FT*-e	34–39*	spleen	+	normal histology	363**
2	-n	-c	34–49	caudal fin	+	fin distorted, projects from body wall at site of implantation.	364
3	-o	-b	34–39	caudal fin	+	as in #2	730***
4	-p	-g	34–39	caudal fin	+	as in #2	374
5	-q	-h	34–39	spleen	+	as in #1	363
6	-s	-i	34–39	caudal fin	+	as in #2	364
7	-t	-j	34–39	caudal fin	+	as in #2	366
8	-u	-k	163–168*	heart, spleen	++	heart beating, both grafts possess normal histology	236
9	-v	-k	163–168	anal fin	−	no trace of graft found in serial sections	236
10	-v	-l	163–168	heart, spleen	++	as in #8	235
11	-u	-l	163–168	anal fin	+	fin imbedded in musculature, normal histology	235
12	-w	-d	163–168	dorsal fin	+	as in #11	237
13	*NJ*-a	*NJ*-b	87	anal fin	+	as in #11	216
14	*DS*-M	*DS*-c	258	heart, spleen	+−	heart beating, no trace of spleen found in serial section	237
15	-M	-D	252	anal fin	+	as in #11	237
16	-α	-H	143	heart, anal fin	++	heart beating, fin as in #11	484
17	-α	-P	93	caudal fin	+	as in #11	237
18	*DS*-AB	*DS*-AE	263	heart, caudal fin	++	as in #16	236
19	*DS*-AC$_\gamma$	*DS*-ACJ	120	heart	+	heart beating, normal histology	236
20	-ACZ	-ACJ	120	caudal fin	+	as in #11	236
21	-AC$_\gamma$	-ACH	122	spleen	+	as in #1	235
22	-ACZ	-ACH	122	anal fin	+	as in #11	235
23	-ACZ	-ACE	127	spleen	+	spleen bright red at graft site	240
24	-AC$_\gamma$	-ACE	127	anal fin	+	as in #2	240
25	-ACZ	-ACA	159	heart	+	as in #19	237
26	-AC$_\gamma$	-ACA	159	caudal fin	+	as in #2	237

* Age of host at time of operation not known exactly, since parent was not monitored for eggs during a five-day period.

** Number of days after the operation at which hosts died or were sacrificed and condition of grafts ascertained.

*** Host still alive and fin graft in excellent condition 730 days after the operation.





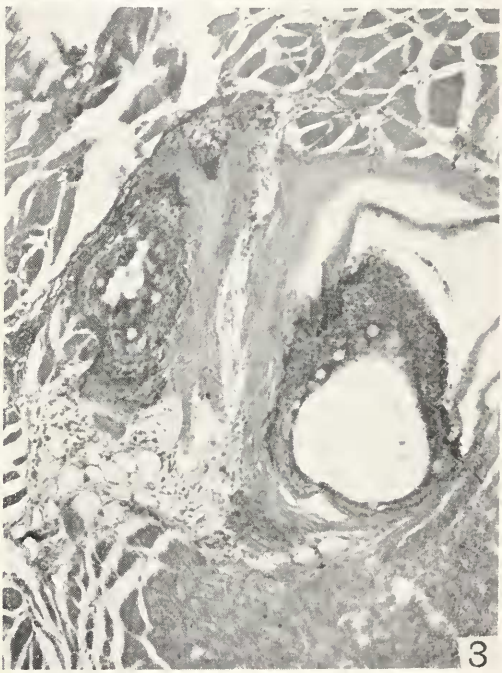


FIGURE 1. Ventral view of *Rivulus* (*NL-AA*) bearing a caudal fin transplant from one of its offspring (*NL-AA*) on its right side slightly above anal fin. Transplanted fin is deformed. Photographed 514 days after the operation. × 2.5.

FIGURE 2. Cross-section through the proximal portion of a caudal fin transplant (*NA-a* → *NA*). The two large cellular bony elements are hypural bones of the basal plate of the caudal fin. The dark acellular bones (circular) are the external fin rays. Notice the loose connective and adipose tissue between the fin rays and the periosteal layer surrounding the hypurals. Host was sacrificed 234 days after the operation. × 180.

FIGURE 3. Cross-section through the distal end of an anal fin transplant (*DS-α* → *DS-H*) which had been deeply inserted into the musculature of the caudal peduncle. No external fin rays regenerated, but inside the skeletal muscle the fin epidermis had rounded up and formed a vesicle. Numerous goblet cells, very typical for *Rivulus*, can be seen in the epidermal layer facing the vesicle. The dermis borders at the musculature. In the proximal region of the graft (not shown here) the other elements of typical fins were found. Host was sacrificed 484 days after the operation. × 180.

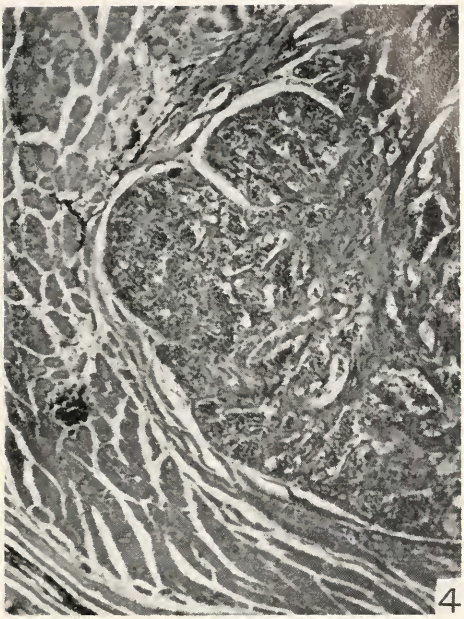
The fate of seven fin transplants could readily be ascertained by macroscopic examination at the time when the hosts were sacrificed. The transplants had grown into typical fins which, however, were distorted as a result of the twisting and injury of the fin rays at the time of the operation (Fig. 1). The fate of nine other fin grafts was verified by histological examination. In these hosts, the fin grafts, which were very small, had been pushed deep into the musculature of the host, and its integument had closed over the mouth of the pocket before the fin could regenerate. Serial sections revealed all the elements of typical fins imbedded in the musculature (Fig. 2). In some cases the fin epithelium of the "external fin" had formed a vesicle (Fig. 3), the inside of which was lined by the fin epidermis with its characteristic mucus cells. Failure to regenerate an external fin in these cases has nothing to do with an immunological reaction. It merely resulted from the fact that the fin had been inserted rather deeply into the pocket.

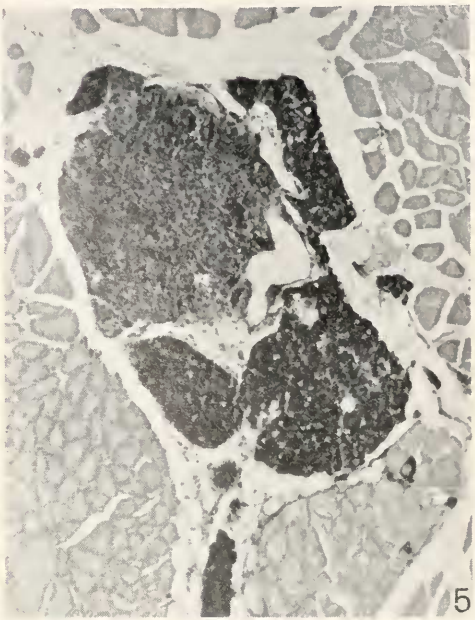
The heart grafts became vascularized within three to four days after the operation and some of them resumed their rhythmic contraction as early as the second day after the operation. All heart transplants were still beating at the time the hosts were sacrificed. Histological examination of all heart transplants failed to reveal any degenerative changes (Fig. 4).

The spleen graft could often be seen through the skin as a dark red structure imbedded in the musculature. The transplanted spleens proved indistinguishable histologically from the host spleens (Fig. 5). It should be noted that two of the hosts, *FT-c* and *FT-d*, had also received a second transplant from a donor belonging to a different line (Table III). These inter-line grafts were rejected. At the end of the experiment all but two of the 31 intra-sib grafts were found. One of these two, host *FT-k*, had received a spleen and a heart graft from *FT-u*, both pushed deeply into the musculature of the caudal peduncle. At the same time, the anal fin of a second donor, *FT-z*, was implanted just anteriorly to the heart and spleen grafts. During the operation the anal fin was damaged. On the seventh postoperative day the fin and the area around the pocket were greatly inflamed and the graft appeared to be disintegrating. Fourteen days after the operation only a few fin rays were seen. There was no indication of any regeneration. The host was finally sacrificed 236 days after the operation. The heart and spleen grafts were present and intact, but no trace of the anal fin could be found. The authors are of the opinion that the failure of this graft to survive is more likely the result of injury during the operation than of immunological reaction. The second exceptional host, *DS-c* (#14), received a heart and spleen graft from *DS-M*, implanted in close proximity. The spleen graft could be observed through the skin for five weeks, but when the fish was sectioned 237 days after the operation, a perfectly normal heart was found but no trace of the spleen. Again the authors share the opinion that this graft failure was not caused by an immunological reaction, but possibly in this case by resorption owing to the extremely small size of the graft.

In Table II are listed six "offspring to the parent" graft combinations. The survival of grafts in this combination is the chief criterion for the occurrence of uniparental reproduction (Kallman, 1962). All six fin grafts survived. Similarly, the two "F_2 into nonparental F_1" combinations were also successful.

Because of a lack of suitable fish, we could only test two "parent to offspring" combinations. This combination can be used to determine whether the parent is





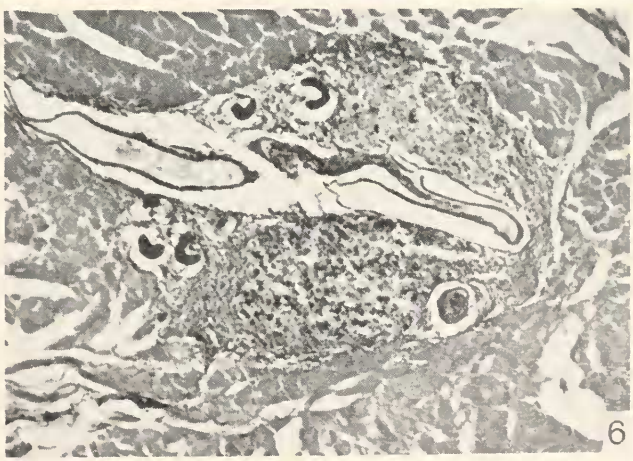


FIGURE 4. Cross-section through part of a heart transplant (*DS-α* ⟶ *DS-H*). Host sacrified 484 days after the operation. × 180.

FIGURE 5. Cross-section through a transplanted spleen to which inadvertently some pancreatic tissue adhered (*NSU* ⟶ *NSU-H*). Host sacrified 234 days after the operation. × 180.

FIGURE 6. Cross-section through the site of a disintegrated caudal fin graft (*NL-AB* ⟶ *NSU-i*). The soft tissue around the fin rays has been completely destroyed and replaced by host connective tissue and histiocytes. Notice the remnants of the fin rays (dark-staining crescent-shaped structures) and the remnants of two disintegrated hypural bones. Host sacrificed 290 days after the operation. × 180.

still heterozygous with different alleles segregating during gametogenesis. If this is so, then certain alleles of the parent may not be represented in the offspring and certain tissue antigens of the parent may be "foreign" to the offspring. The offspring, therefore, may reject parental tissue grafts. In our two combinations, both grafts survived.

In order to determine whether any of the six wild-caught *Rivulus* were members of the same clone, we performed eight interline grafts in seven out of 16 possible host donor combinations (Table III). Of these, a caudal fin graft from an *NSU*

TABLE II

Fate of intraline grafts in Rivulus marmoratus *between hosts and donors belonging to different generations*

#	Donor	Host	Age of host	Type of graft	Fate of graft	Criteria of graft survival	Time (days)
				Offspring to Parent Grafts			
27	*FT*-X	*FT*		caudal fin	+	fin imbedded in musculature, normal histology	355*
28	-W	*FT*		anal fin	+	as in #27	234
29	*NA*-a	*NA*		caudal fin	+	as in #27	234
30	*NL*-AA	*NL*-A	407	caudal fin	+	fin projects from body wall	613
31	-BA	-B	407	caudal fin	+	as in #27	220
32	*NSB*-AA	*NSB*-A	266	caudal fin	+	as in #27	234
				Parent to Offspring Grafts			
33	*NSU*	*NSU*-B	73	spleen	+	normal histology	235
34	*NSU*	*NSU*-H	507	spleen	+	normal histology	234
				F_2 into Nonparental F_1			
35	*NL*-AB	*NL*-B	535	anal fin	+	fin distorted, projects from body wall at site of implantation, normal histology	92
36	*NSB*-AA	*NSB*-C	266	caudal fin	+	as in #35	241

* Number of days after the operation at which hosts died or were sacrificed and condition of graft ascertained.

survived in a *DS* host, indicating that *NSU* and *DS* and their descendants belong to the same clone. All other transplants disintegrated. The caudal fin from the *NL* donor implanted in an *NSU* host exhibited the chronic type of graft rejection. When the host was fixed 290 days after the operation and histologically examined, remnants of the disintegrated graft were still observed (Fig. 6). The chronic rejection of tissue grafts is usually found in cases where host and donor differ by only a few histocompatibility loci. Since in uniparentally reproducing species new clones arise by the accumulation of mutations, the existence of clones differing from each other by few genes is to be expected.

Discussion

These results are in agreement with the conclusion that these fish are self-fertilizing hermaphrodites (Harrington, 1961, 1963). Since self-fertilization represents the ultimate in inbreeding, these fish must have achieved a high degree of homozygosity in which identical alleles segregate during gametogenesis and the offspring possess a genotype identical with that of the parent.

Billingham and Silvers (1959) have pointed out that the most sensitive indicator for homozygosity is the tissue transplantation test; Kallman (1962) has discussed its application to the study of uniparental reproduction in vertebrates. The test is predicated on the fact that in vertebrates tissue transplants from one individual to another of the same species only succeed if all, or almost all, of the donor's antigens are also present in the host. The presence or absence of tissue antigens is under the control of specific genes, called histocompatibility genes (for reviews see Snell, 1957; Medawar, 1959; Owen, 1959). The test is valid only if a large number of genes are concerned with transplantation immunity, because otherwise

Table III

Fate of interline grafts in Rivulus marmoratus

#	Donor	Host	Age of host	Type of graft	Fate of graft	Criteria of graft survival	Time (days)
I	*NSU*-C	*DS*-AC	289	caudal fin	+	fin imbedded in musculature, normal histology	237*
II	*FT*-a	*DS*-A	506	heart, spleen	− −	no trace of grafts found in serial sections	240
III	*NL*-a	*DS*-ACF	125	spleen	−	as in #II	234
IV	*NSU*-F	*FT*-b	163	caudal fin	−	as in #II	376
V	*NL*-AB	*FT*-c	163	heart	−	as in #II	236
VI		died					
VII	*NL*-BA	*FT*-d	163	anal fin	−	scar tissue present	365
VIII	*NL*-AB	*NSU*-i	504	caudal fin	−	scar tissue present	290
IX	*NL*-AB	*NSB*-B	266	dorsal fin	−	as in #II	377

* See Table II.

two individuals selected at random might be identical with respect to their histocompatibility genes, yet heterozygous and thus different with respect to many other loci. For this test to be valid, the histocompatibility genes must also exist at least in two or more allelic states and be scattered randomly over the chromosomes. If these conditions are fulfilled, the chances that two individuals selected at random from a large interbreeding population would have compatible genotypes are extremely small. In the mouse the number of histocompatibility genes has been estimated to be at least 13–16 (Barnes and Krohn, 1957; Prehn and Main, 1958), and a similar number has been reported for the rat (Billingham *et al.*, 1962). In the teleost, *Xiphophorus maculatus*, at least twelve histocompatibility genes have been demonstrated (Kallman, unpublished data).

The low estimates of four to six histocompatibility genes for the guinea pig (Bauer, 1960) and only three for the Syrian hamster (Billingham *et al.*, 1960) should be considered in the context of the unitary origin of the strains employed. All laboratory strains of the Syrian hamster can be traced back to two females and

a single male belonging to a litter of twelve captured in 1930. The hamster strains that have come into existence during the last 33 years probably have been created in the same way as many other laboratory strains. They have been founded by one, two or three breeding pairs obtained from colonies existing elsewhere. That this founder principle has led to a general decay of the genetic variability, accompanied by the fixation of certain alleles and loss of others through genetic drift, is most likely. Similar results indicating a small number of histocompatibility genes have recently been reported by Billingham and Silvers (1963) for another subspecies of the hamster, *M. a. brandti*. Without additional experiments, however, it is still premature to conclude that hamsters, in contrast to mice, rats and platyfish, have only a few transplantation antigens. Wild-caught individuals are usually considered heterozygous for a large number of loci, but such an assumption may not always be justified and it has yet to be shown just how heterozygous hamsters are in nature. If the reports are true that the hamster is rather uncommon, then the effective breeding population within a particular area will be relatively small, resulting in a certain degree of homozygosity. In this case, even the survival of some skin transplants exchanged among sibs of wild-caught parents could be understood. A strikingly similar situation has recently been described for two disjunct populations of the platyfish, *Xiphophorus couchianus* (Kallman, 1964). A high percentage of transplants exchanged among the offspring of wild-caught females were permanently accepted, provided the females had been collected in springs where the population density of this species was lowest. Similar results have been obtained with certain wild-caught *X. variatus* and *X. maculatus* (Kallman, unpublished).

No valid estimate of the number of histocompatibility genes has been presented for any other species, but since it is almost the universal experience of biologists that homotransplants exchanged among wild-caught animals of the same species or among members of heterozygous strains are rejected, it is likely that large numbers of histocompatibility genes are present in most species. Tissue transplantation experiments have been performed in twelve species of teleosts (for references see Kallman, 1964), all wild-caught or heterozygous stocks, but not a single transplant survived with the exception of those in *X. couchianus*, *X. variatus* and *X. maculatus*.

The transplantation test, therefore, is valid and the survival of almost all intra-line grafts in *Rivulus* can be taken as excellent evidence that the descendants of each wild-caught fish are identical genetically and constitute a clone. No segregation of histocompatibility genes had taken place and therefore the fish were presumably homozygous not only for these genes but for most of the genome. The last point, of course, cannot be settled with absolute finality, because of the logical, though biologically remote, possibility of an unknown mechanism that keeps these fish permanently heterozygous through successive generations. Conceivably such a mechanism would entail the nonviability of all homozygotes, but this possibility seems highly unlikely, since under optimum laboratory conditions, at least, with intensive monitoring of eggs, the number of eggs failing to develop was low. There is also the possibility that the eggs may develop gynogenetically after being activated by the fish's own sperm. This question will have to be settled, eventually, through cytological analysis.

The prevalence of normal development in *Rivulus* is no serious obstacle to the view that these fish are homozygous. Homozygosity would have been gradually achieved with enough time for the elimination of lethal and deleterious genes, in contrast to meiotic parthenogenesis in which homozygosity may be achieved in a single step. Salekhova (1963) reported that in *Serranus scriba,* artificially self-fertilized eggs develop normally, but better survival was obtained in cross breeding.

Kallman (1962, 1963) showed that natural populations of *M. formosa* consist of a number of clones, the frequencies of which remained rather constant from year to year. It is very likely that a similar condition will be found in *Rivulus.* Although the results of our clonal analysis are preliminary because of the small numbers of fish, they show that at least two fish derived from parents collected in different places evidently belong to the same clone. Of these parents, *DS* and *NSU* (*cf.* Table III for their descendants used as host and donor), one was collected June 13 and the other June 24, 1960, the first to the north and the second to the south of a long-established road running due east to the open lagoon and thus forming a barrier between north and south expanses of the marsh. Each fish would be trapped in the depression of the march where it was caught, except when high tides and heavy rains flood the marsh, which is only during the autumn months. The effective distance between the two fish then would involve movement east to the lagoon shore, north or south along the lagoon to pass the road barrier, and west in the other marsh expanse, 420 + 405 + 375 yards, or *vice versa.* In sum, a minimum route of 1300 yards, open in its entirety only one season of the year, would have to be traversed by a fish travelling between these two collecting sites.

It is interesting to determine whether other vertebrates that normally reproduce uniparentally are also homozygous. In the gynogenetic cyprinid, *Carassius auratus gibelio,* the egg nucleus is in the metaphase of the second meiotic division when the eggs are deposited and fertilization takes place (Lieder, 1959). Forty minutes later the second polar body is extruded. The female pronucleus possesses the haploid chromosome number, but before the first cleavage division is completed, the diploid condition has been reestablished. Lieder (1959) could not determine how this is accomplished, but states that there is no fusion of the egg nucleus with the second polar body. The diploid condition may therefore be reestablished by chromosome doubling without cell division and the fish, consequently, would be homozygous. It is not possible to determine with the transplantation test whether the gynogenetic teleost, *M. formosa,* is homozygous or heterozygous (Kallman, 1962). Regardless of whether the fish arises from haploid or diploid eggs, parent and offspring possess identical genotypes and a "three-way histocompatibility" among parent and offspring exists.

On the basis of a few cytological preparations it appears that individuals of the parthenogenetic subspecies of the lizard, *Lacerta saxicola,* are also homozygous. Darewski and Kulikowa (1961) state that the first meiotic division is normal and that the diploid condition is reestablished either as a result of the fusion of the products of the second meiotic division or the suppression of the first cleavage division. Both processes result in homozygosity.

Several sporadic cases of uniparental reproduction in vertebrates have to be considered "abnormal" and these offspring all appear to be heterozygous. No transplantation experiments were performed with Spurway's guppies, which are

produced by self-fertilization, but a segregation of pigment genes among the uniparental offspring clearly indicated that they were heterozygous as expected in a first, "selfed" generation.

The heterozygous nature of male parthenogenetic turkeys was proven by transplantation tests. Healey *et al.* (1962) showed that there existed only a "one-way histocompatibility" between parthegenones and parent; grafts from the offspring survived in their respective parents, but all parental grafts were rejected by the offspring, as were grafts between parthenogenetic sibs. Chromosome segregation, therefore, had taken place and parent and offspring did not possess identical genotypes. The question whether the parthegenones were homozygous or heterozygous was settled by an additional transplantation experiment (Poole *et al.*, 1963). Offspring sired by the parthegenones rejected grafts from their male parent. This clearly indicated that the parthegenones were heterozygous.

In *Gambusia affinis* a similar "one-way histocompatibility" between parthenogenetic offspring and parent was observed by Kallman and Kilby (unpublished). Only grafts from offspring to parent survived. Whether these fish were homozygous or heterozygous could not be determined since the parthegenones failed to reproduce.

Summary

1. Since self-fertilization constitutes the ultimate basis of inbreeding, a species or race of self-fertilizing hermaphrodites should consist of clones, all members of which possess identical genotypes and are homozygous. This prediction has been tested on a recently discovered population of *Rivulus marmoratus* from the east coast of Florida. All wild-caught specimens of this population tested so far have proved to be hermaphrodites. Those kept in isolation in aquaria, and their hermaphrodite descendants kept in isolation *ab ovo*, have reproduced by self-fertilization.

2. The transplantation test has been used to determine whether fish that had descended from the same wild-caught progenitor possess identical genotypes. Fins, spleens and hearts were transplanted in 36 different host-donor combinations involving six different lines (sib to sib, parent to offspring, offspring to parent). Only two transplants failed to survive, but their loss may have been due to mechanical reasons. These results are in accordance with the theoretical prediction that these fish are largely homozygous.

3. Transplants were performed in seven different inter-line combinations. Of these only a single graft survived, indicating that the fish collected on different days in different places belonged to the same clone.

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